CROP PHYSIOLOGY & METABOLISM

Nitrogen and CO_2 Affect Regrowth and Biomass Partitioning Differently in Forages of Three Functional Groups

Jack A. Morgan,* R. Howard Skinner, and Jon D. Hanson

ABSTRACT

Little work has been done to assess the impact of elevated CO_2 on responses of forages to defoliation. This study examines regrowth, biomass partitioning, and labile C and N metabolites in three functional plant-types: a C_3 grass [*Pascopyrum smithii* (Rydb.) A. Love], a C_4 grass [*Bouteloua gracilis* (H.B.K.) Lag.], and a forage legume (*Medicago sativa* L.). Plants were grown from seed, defoliated twice, and grown in a controlled environment under a factorial arrangement of two CO_2 [low CO_2 (LC), 355 μmol mol^{-1}, and high CO_2 (HC), 700 μmol mol^{-1}] and two N nutrition regimes [low N (LN), watered twice weekly with half-strength Hoagland's containing 0 N, and high N (HN), half-strength Hoagland's containing 14 m*M* N]. High N enhanced regrowth in all three species, while high CO_2 enhanced regrowth only in the two C_3 species. In *M. sativa*, CO_2 and N treatments had no significant effect on *k*, the allometric growth coefficient. In contrast, *k* was reduced in *P. smithii* plants grown under LN (0.63) compared with HN (0.99). In *B. gracilis*, low N also reduced *k*, but it interacted with CO_2 so that *k* was greatest for plants grown at HN/HC (0.95) and HN/LC (0.89), intermediate at LN/LC (0.58), and least at LN/HC (0.44). These results indicate greater partitioning to belowground organs (reduced *k*) when N is limiting, particularly under elevated CO_2. Significant correlations were established between *k* and several measures of plant N status, suggesting that the effects of CO_2 on plant biomass partitioning involve N status.

DESPITE THE IMPORTANCE of the effect of grazing pressure on the production and ecology of rangelands and pastures (Harris, 1978; Milchunas and Lauenroth, 1993), only a limited amount of attention has been given to studying how CO_2 enrichment may interact with defoliation. Carbon dioxide enrichment studies with forages have often included defoliation as a component (Bunce, 1995; Campbell and Hart, 1996; Lüscher et al., 1996; Owensby et al., 1999; Schenk et al., 1997), but few have explicitly evaluated how defoliation interacts with CO_2 to effect plant productivity. Growth of frequently (Ryle and Powell, 1992) or periodically (Sæbø and Mortensen, 1995) defoliated white clover (*Trifolium repens* L.) was significantly enhanced (30–45%) in plants grown at twice ambient CO_2 concentrations, but forage regrowth of several grass species was affected little or reduced (to the point of inhibition in some cases) at elevated CO_2 (Hunt et al., 1995; Sæbø and Mortensen, 1995; Wilsey, 1996). Repeated defoliation decreased CO_2-induced growth enhancements in grasses (Hunt et al., 1995) and in *Lolium perenne* L. (Hebeisen et al., 1997) under nutrient-limited conditions. However, when nutrients were abundant, positive CO_2-induced growth enhancements were maintained under defoliation. These results indicate the importance of soil nutrients in sustaining CO_2-induced growth responses of forages, especially when subject to defoliation.

The partitioning of biomass between above- and belowground organs in response to environmental perturbations like CO_2 is another important plant trait that has been studied extensively, but little information exists on how defoliation might interact with CO_2 and affect partitioning. The enhanced plant growth that generally occurs under elevated CO_2 concentrations is often accompanied by shifts in biomass partitioning, often (although not always) with increased partitioning of biomass to belowground organs (Bazzaz, 1990; Rogers et al., 1994, 1996). Several researchers (Campagna and Margolis, 1989; Hunt et al., 1998; Stulen and den Hertog, 1993) have suggested that such CO_2-induced changes in plant biomass partitioning among organs may be best understood through an examination of the effects on labile C and N pools and the functional balance hypothesis. This hypothesis, proposed by Davidson (1969), states that partitioning of biomass among plant organs results from a plant's tendency towards functional balance, a state in which root/shoot activities are adjusted towards maintenance of a stable C/N ratio.

A large body of knowledge exists describing different plant species responses to elevated CO_2, with some progress toward identifying different plant functional groups in terms of their potential responses to CO_2. Plants possessing the C_3 photosynthetic pathway are generally expected to exhibit a greater growth enhancement from CO_2 enrichment since their photosynthetic apparatus is limited more by present ambient CO_2 concentrations compared with C_4 plants (Bowes, 1991). Evidence from numerous studies indicates that this expectation holds (Bazzaz, 1990; Poorter, 1993; Wand et al., 1999), although it can be complicated by other aspects of plant/soil responses to CO_2, as well as interactions of plant CO_2 responses with other environmental parameters (e.g., Hunt et al., 1996, Owensby et al., 1993, 1999).

Jack A. Morgan, USDA, ARS Rangeland Resources Research Unit, 1701 Centre Ave., Fort Collins, CO 80526; R. Howard Skinner, USDA, ARS, Pasture Systems and Watershed Management Research Laboratory, Bldg. 3702 Curtin Road, University Park, PA 16802; Jon D. Hanson, USDA, ARS, Northern Great Plains Research Laboratory, PO Box 459, Mandan, ND 58554. Received 4 Feb. 2000. Corresponding author (morgan@lamar.colostate.edu).

Published in Crop Sci. 41:78–86 (2001).

Abbreviations: C_{lab}, labile C compounds; DW, dry weight; HC, high CO_2; HN, high N; *k*, allometric growth coefficient; LC, low CO_2; LN, low N; N_{lab}, labile N compounds; SDW, structural dry weight; TNC, total nonstructural carbohydrates.

In one of the few studies to evaluate differences among species from different photosynthetic functional groups in how CO_2 enrichment affects regrowth and plant partitioning, Wilsey et al. (1997) observed significant growth enhancements and increased partitioning of plant biomass to storage organs from CO_2 enrichment in growth chamber studies of North American C_3 grasses, but no CO_2 responses in C_3, C_3/C_4 intermediate, and C_4 grasses from Argentina or Tanzania.

Differences in the capability to fix atmospheric N may also determine a species' capacity to respond to atmospheric CO_2 enrichment. Growth of N-fixing species may be more responsive to elevated CO_2 given their additional C sink resulting from N fixation plus the added available N generated; these two factors should increase the capacity and activity of a plant's photosynthetic apparatus, and therefore its capability to respond to CO_2 enrichment. In fact, legumes often do exhibit superior growth response to CO_2 enrichment in N-limited systems compared with associated grasses (Hebeisen et al., 1997; Luscher et al., 1996; Sæbø and Mortensen, 1995; Schenk et al., 1997), although cool temperature can shift the balance towards cool-season grasses (Campbell and Hart, 1996).

This study evaluates regrowth in three forages of contrasting functional groups, *Pascopyrum smithii* (C_3 native perennial grass of the Great Plains), *Bouteloua gracilis* (C_4 perennial grass of the Great Plains) and *Medicago sativa* (C_3 perennial forage legume) in the 20 d following a defoliation event under two N and CO_2 regimes. We hypothesized that, due to differences in photosynthetic pathway and N-fixing capability, stimulation of regrowth by elevated CO_2 would be greatest in *M. sativa* and least in *B. gracilis*, especially under the low N-fertility regime. We also evaluated how CO_2 enrichment affected the partitioning of biomass in defoliated species in relation to the balanced-growth or functional equilibrium concept (Davidson, 1969). Our primary interest was to determine how tissue concentrations of labile C and N metabolites and their relative proportions to each other were affected by different CO_2 and N treatments, and how variability in these labile metabolites related to biomass partitioning in the 10 to 20 d after defoliation. Since elevated CO_2 often leads to reduced tissue N concentrations, we hypothesized that the treatment combination of elevated CO_2 and low soil N availability would likely result in the lowest tissue N concentrations, particularly in the two grass species, and would lead to increased partitioning of biomass to belowground organs.

MATERIALS AND METHODS

Plant Culture

Polyvinylchloride cylinders 15-cm diameter by 45 cm deep were filled with a 50:50 mixture of sand and prairie soil [Remit fine sandy loam (Ustollic camborthids)]. Seeds of *M. sativa*, *B. gracilis*, and *P. smithii* were sown in the soil mixture and the columns were placed in a greenhouse for establishment at ambient CO_2 and daytime temperatures of approximately 25°C. After germination, seedlings were thinned to two per column, and N treatments were begun. Columns received biweekly additions of half-strength Hoagland's solution, with half of the columns receiving 0 (LN) and the other half 14 (HN) m*M* N as NH_4NO_3. As the seedlings grew, columns were irrigated with water as needed to avoid water stress and to flush nutrients through the soil.

Six weeks after emergence, established plants were defoliated with shears to a 5-cm height and columns transferred to an EGC walk-in growth chamber (Environmental Growth Chambers, Inc., Model No. M11, Chagrin Falls, MI, USA)[1] for conditioning to CO_2 regimes. Chambers were maintained at either 355 (ambient or LC) or 700 (elevated or HC) μmol mol^{-1} CO_2, a 14-h photoperiod, 28/17°C day/night temperature regime, 700 μmol m^{-2} s^{-1} photosynthetic photon flux density, and a daytime vapor pressure deficit of 1.7 kPa. The environmental conditions were similar to those observed on the Colorado shortgrass steppe in the summer (Lauenroth and Milchunas, 1991), and were chosen because of the importance of this time of year for cattle grazing. After 3 wk of regrowth in the growth chambers, all plants were defoliated a second time to a 5-cm height. For the grasses, this cutting height removed all mature leaf blades, leaving only sheaths and enclosed elongating laminae. *P. smithii* is rhizomatous, so in addition to shoot growth arising from the main stem and crowns of the two selected plants, additional shoots were initiated by rhizomes. Any such rhizomatous growth was excised at the soil surface. Any unfolded *M. sativa* leaves remaining below the 5-cm cutting height were also removed. After this second defoliation, the following biomass and metabolite samples were taken.

Biomass and Metabolites

Sequential harvests made on different columns at 0, 4, 7, 10, 14 and 20 d after cutting were made to analyze how regrowth varied among the three species because of growth under different N and CO_2 regimes. Plants were separated into roots (including rhizomes for *P. smithii*), crowns, and shoots (regrowth), and lyophilized in a freeze drier. In the grasses, mature sheaths below the 5-cm cutting height were included with crowns, while elongating leaves within sheaths were placed with the regrowth. New emergent tillers were excised at the soil surface and placed with regrowth. The distal 5 cm of each leaf blade that was elongating at the time of cutting was included with the crowns since those tissues were part of the stubble left after clipping. *Medicago sativa* regrowth included all leaves and stems above a 5-cm height plus all unfolded leaves below that height. Biomass of each organ was analyzed and partitioned into N compounds, nonstructural carbohydrates, and structural biomass [organ dry matter − (nonstructural carbohydrates + labile N compounds)] according to Skinner et al. (1999).

Partitioning Analysis

To examine treatment effects on partitioning of structural biomass between above- and below-ground organs (Troughton, 1956), the allometric growth coefficient (k) was determined for each species according to the following equation:

$$1n\ S = 1n\ b + k\ 1n\ R$$

where S is shoot structural biomass, R is root structural biomass, and b is a constant. If $k = 1$, then relative root and shoot growth are equal. If $k < 1$, then relative root growth > shoot growth, and when $k > 1$, then relative root growth < shoot growth. The estimates were obtained from harvests per-

[1] Mention of a trademark, proprietary product, or vendor does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products or vendors that also may be suitable.

formed 10 to 20 d after defoliation. The first three harvest dates were excluded from analysis because mobilization of labile reserves was active during this period (Skinner et al., 1999); this complicated the analysis of root:shoot partitioning responses, which are better represented by more stable, steady-state conditions. Further, a lack of significant shoot biomass immediately following defoliation compromised our ability to detect accurately labile compounds as well as determine shoot biomass. The allometric growth coefficient *k* represents the balance between shoot and root relative growth rates, and so provides an estimate of treatment effects on partitioning that is not confounded by ontogenetic attributes like plant size (Coleman et al., 1993; Farrar and Williams, 1991). Calculation of a structural *k* is presumed to reflect more accurately longer-term partitioning responses, unconfounded by the more short-term build-up of labile C and N compounds in tissues.

We evaluated several measures of whole-plant C and N status and correlated these measures against *k* to determine how such measures related to the growth coefficient of recently defoliated plants. In particular, we evaluated whole-plant concentrations of total non-structural carbohydrates (TNC), labile N compounds (N_{lab}), total N, N_{lab} as a percentage of total plant N, and ratios of various C and N compounds. The N_{lab} fraction consists mostly of NH_4, NO_3, and free amino acids. The analysis of N_{lab}, where it is referred to as low molecular weight N compounds, plus the N and TNC analyses are described in Skinner et al. (1999).

Statistics

Since only one growth chamber was available for this study, the experiment was repeated four times, two times at 350 μmol mol^{-1} CO_2 and two at 700 μmol mol^{-1} CO_2. For each of the four runs, seedlings were established in the greenhouse and procedures followed as previously described before and after transfer to the growth chamber. Within each run, a factorial arrangement of three species, two N concentrations, six harvest dates, plus three subsamples (not counted as replicates) for each factorial combination were randomly arranged within the growth chamber for a total of 108 columns. Therefore, the experiment was analyzed as a split-plot experiment with two replications. The CO_2 treatments which consisted of the four separate runs were treated as the whole plots, and were completely randomized. Because responses varied considerably among the species, separate analyses were conducted for each species. Fishers least significant difference means comparison test was used to test for differences among treatment means when the *F* test indicated significance ($P \leq 0.05$). Correlations calculated between the growth coefficient (k) and measures of plant C and N status were performed with treatment means.

RESULTS AND DISCUSSION

Regrowth

Medicago sativa shoot regrowth was enhanced by both N and CO_2 (Table 1; N and CO_2 effects). Effects of both CO_2 and N on shoot and whole plant regrowth became greater with time (Table 1; D × C and D × N interactions), so at 20 d both shoot and whole-plant regrowth was greatest in plants grown at high N and high CO_2 (HN/HC), lowest for plants grown at LN/LC, and intermediate for the other two combinations of N and CO_2 (Fig. 1 and 2). Similar treatment separations were seen in crown and root dry weights (Fig. 3 and 4). Root and crown regrowth was initially greatest for plants grown under HN/HC, and then appeared to both decrease and then recover sooner than the other treatments in response to defoliation. These responses may have been due in part to mobilization of reserve compounds, as elevated CO_2 increased both the rate and total amounts of TNC for mobilization in *M. sativa* (Skinner et al., 1999). However, regrowth in completely defoliated plants relies totally on storage compounds for only a few days, and then depends increasingly on metabolites generated from the newly formed photosynthetic surfaces (Richards and Caldwell, 1985). Nodules were apparent on roots of *M. sativa* at all harvests. Although not measured, the nodules appeared noticeably larger and more abundant under elevated CO_2, indicating that enhanced regrowth may have been due in part to increased N fixation (Zanetti et al., 1996). Stimulation of photosynthesis was also a likely contributor to enhanced growth at elevated CO_2 (Bazzaz, 1990; Bowes, 1991).

As expected, the summer-like conditions in the growth chamber were less favorable for regrowth in *P. smithii* compared with *B. gracilis* and *M. sativa*. By 20 d after defoliation, total dry weights of *P. smithii* plants averaged across all treatments were only 28% of values observed for *B. gracilis* plants (Fig. 2). In contrast to

Table 1. Analysis of variance results for growth of *M. sativa, P. smithii* and *B. gracilis* whole plants and organs for six dates (D; 0, 4, 7, 10, 14, and 20 d after defoliation), two nitrogen (N) regimes (0 and 14 m*M* N in half-strength Hoagland's watered twice weekly), and two CO_2 (C) environments (350 and 700 μmol mol^{-1}).

		Treatments						
Species	**Trait**	**Date**	**N**	**CO_2**	**D × N**	**D × C**	**N × C**	**D × N × C**
M. sativa	**shoot dw**	***	***	**	***	***	**NS**	**NS**
	plant dw	***	***	**0.06**	***	***	**	**NS**
	crown dw	***	***	**NS**	**0.06**	**0.08**	**	**NS**
	root dw	***	***	**NS**	*	*	**	**NS**
P. smithii	**shoot dw**	***	***	**NS**	***	***	***	***
	plant dw	***	***	**NS**	***	***	***	**NS**
	crown dw	***	***	**NS**	***	***	***	**NS**
	root dw	***	**	**NS**	**0.06**	*	**NS**	**NS**
B. gracilis	**shoot dw**	***	***	**NS**	***	***	**NS**	**
	plant dw	***	***	**NS**	***	***	**NS**	**
	crown dw	***	***	**NS**	***	**NS**	**0.07**	**NS**
	root dw	***	**	**NS**	***	***	**0.08**	**0.08**

***, **, and *** indicate significant treatment effect at $P \leq 0.05$, 0.01 and 0.001 respectively. *P* values given between 0.05 and 0.10, otherwise NS ($P > 0.10$).**

M. sativa, differences in *P. smithii* shoot (Fig. 1) and whole plant (Fig. 2) dry weights due to CO_2 were considerably more evident for plants grown at HN. This is consistent with work involving cool-season grasses in which CO_2-induced growth responses were greatest under high N fertility (Hebeisen et al., 1997; Hunt et al., 1995; Wilsey, 1996). Like *M. sativa*, crown dry weights of the HN/HC treatment were initially greater, and generally remained higher throughout the 20 d, suggesting an advantage for regrowth of this treatment. However, mobilization of carbohydrate reserves in this species was unaffected by CO_2 treatment (Skinner et al., 1999). Thus, treatment differences in regrowth for this species appear to be driven primarily by increased C fixation.

Similar to results obtained for *P. smithii* and *M. sativa*, N treatment significantly enhanced regrowth of *B. gracilis* (Table 1). Unlike the C_3 species, elevated CO_2 reduced regrowth at HN in this C_4 grass (Fig. 1 and 2, Table 1; D × N × C interaction).

The reduction in regrowth in *B. gracilis* at elevated CO_2 is curious. Elevating CO_2 above present ambient concentrations is often thought to have little direct effect on the photosynthetic metabolism in C_4 species, although small photosynthetic enhancements have been observed in *B. gracilis* when when CO_2 concentrations were doubled (LeCain and Morgan, 1998; Morgan et al., 1994). There is little information concerning why regrowth in a C_4 grass should be significantly reduced under CO_2 enrichment. Defoliation may decrease the capability of forages to respond to CO_2 (Hebeisen et al., 1997; Schapendonk et al., 1997). This could result simply from the removal of the photosynthetic surface

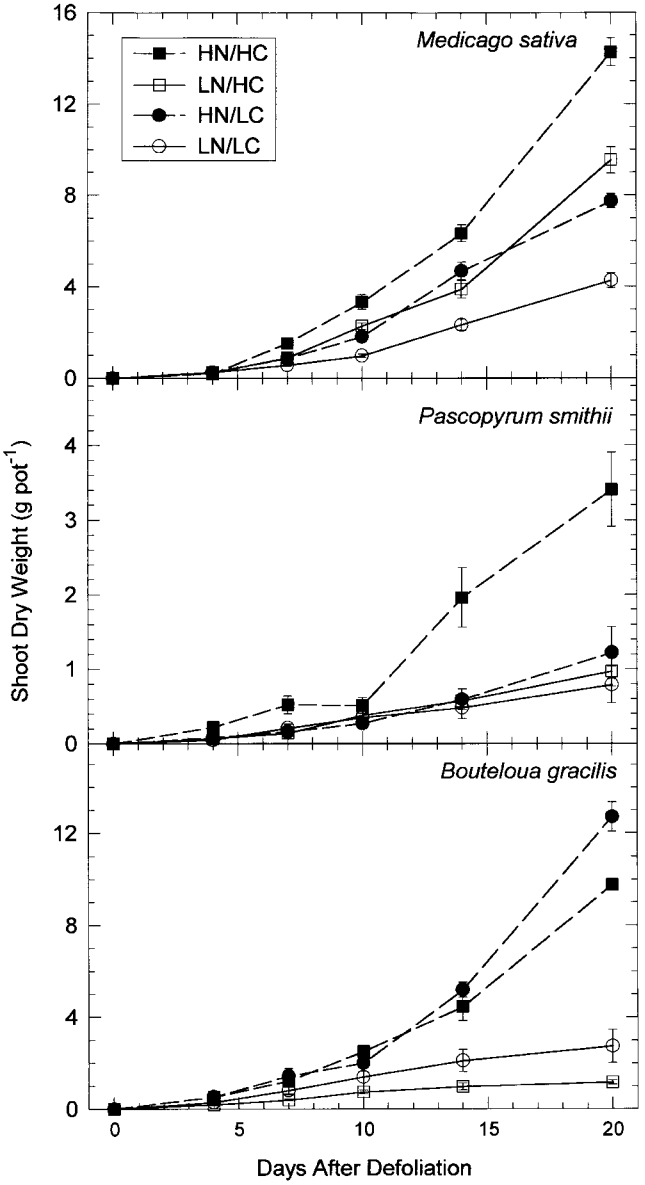

Fig. 1. Changes in shoot dry weight of *M. sativa*, *P. smithii*, and *B. gracilis* when grown under high/low (H/L) CO_2 and N regimes during the 20 d following defoliation. The vertical bars represent ± standard error of the mean.

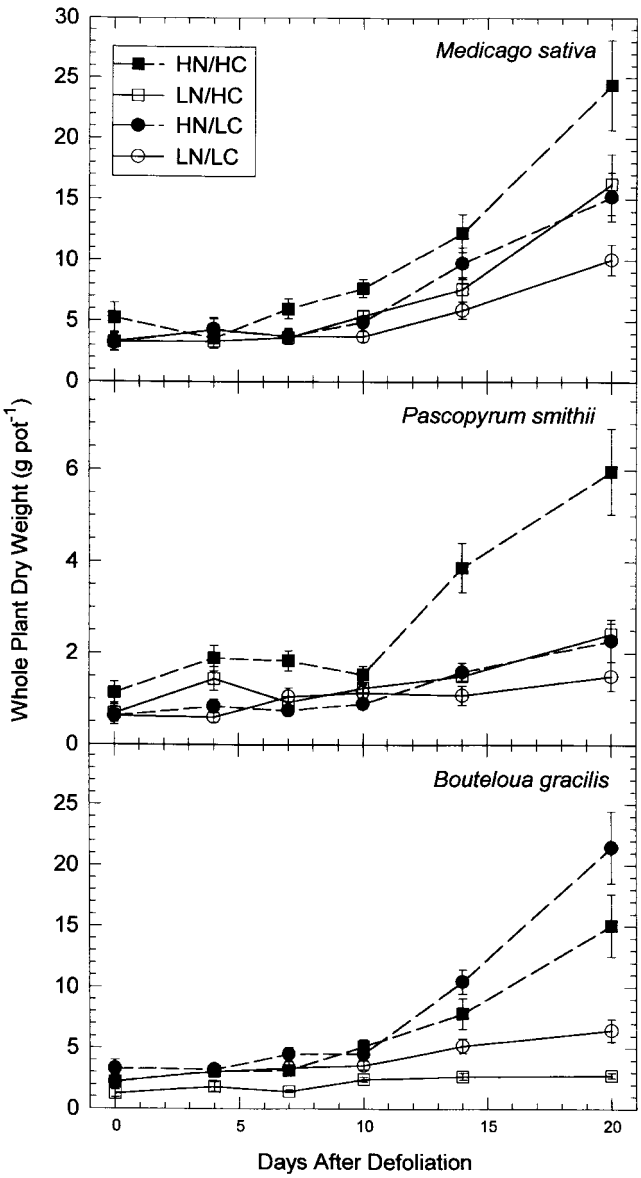

Fig. 2. Changes in whole plant dry weights of *M. sativa*, *P. smithii*, and *B. gracilis* when grown under high/low (H/L) CO_2 and N regimes during the 20 d following defoliation. The vertical bars represent ± standard error of the mean.

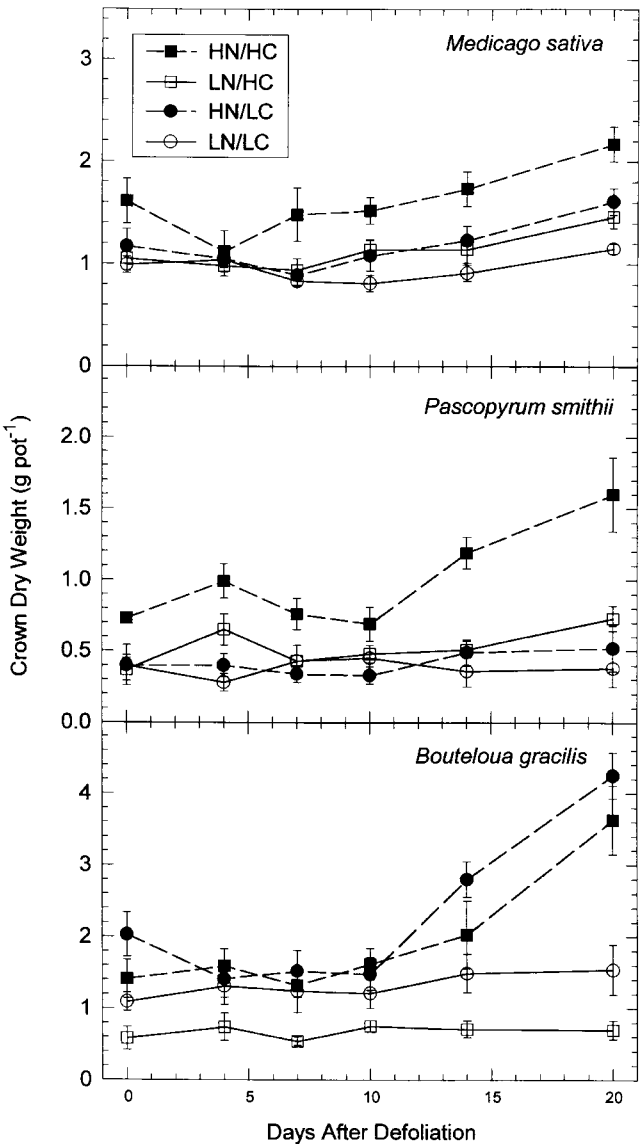

Fig. 3. Changes in crown dry weight of *M. sativa*, *P. smithii*, and *B. gracilis* when grown under high/low (H/L) CO_2 and N regimes during the 20 d following defoliation. The vertical bars represent ± standard error of the mean.

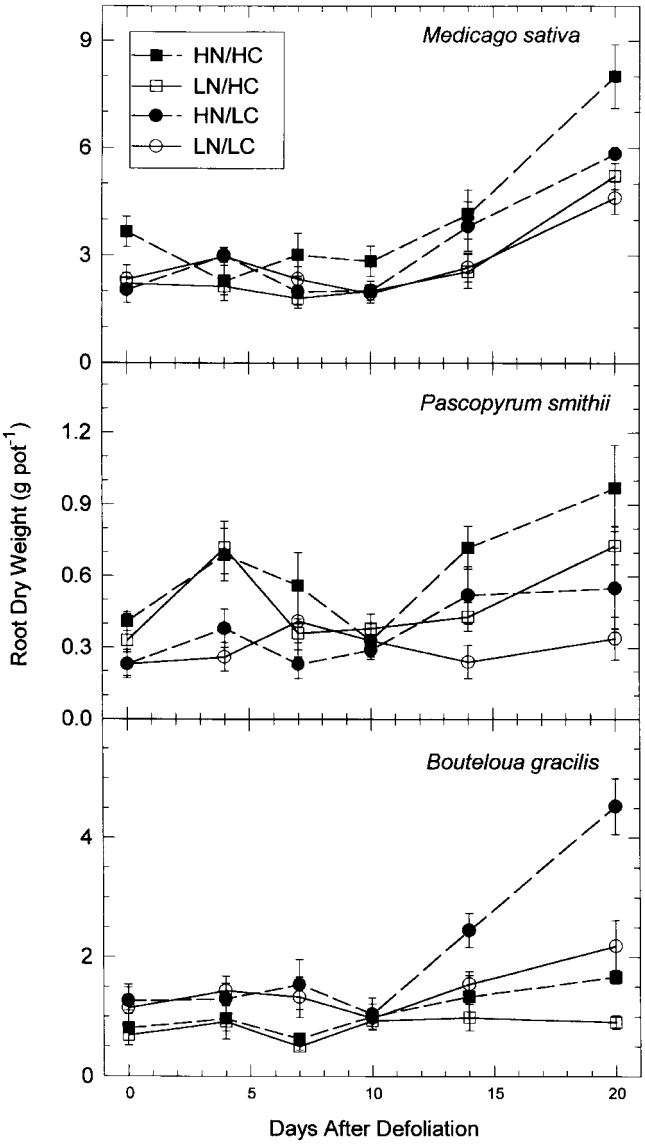

Fig. 4. Changes in root dry weight of *M. sativa*, *P. smithii*, and *B. gracilis* when grown under high/low (H/L) CO_2 and N regimes during the 20 d following defoliation. The vertical bars represent ± standard error of the mean.

upon which the CO_2-induced growth response depends. In the case of frequently defoliated plants, formation of smaller tillers may reduce CO_2 responses through a reduction in sink strength (Hebeisen et al., 1997). But it may also be due to lowered respiration under elevated CO_2. Regrowth of defoliated vegetation is initially driven solely by respiration in the mobilization and utilization of reserves. Numerous studies have shown that elevated CO_2 reduces specific respiration rates in plants (Amthor, 1997; Gonzàlez-Meler et al., 1996; Wullschleger et al., 1994). Although there is little information on how elevated CO_2 might affect respiration involved in plant regrowth, reduced respiration may sometimes down-regulate regrowth processes analogous to elevated CO_2 down-regulating photosynthesis. This may have especially important consequences for C_4 species with less potential to respond positively to CO_2 than C_3 species, and may explain why in the present study only *B. gracilis* exhibited reduced regrowth at elevated CO_2.

Biomass Partitioning

For all species, root:shoot ratio decreased during the regrowth period (Fig. 5; calculated with shoots' including crown tissues), a consequence of mobilization of reserves for above-ground growth, and restoration of the photosynthetic tissues. Root:shoot ratios were sometimes greater under the LN regime. In *M. sativa*, greater root:shoot ratios under LN were detected only in plants grown at LC ($P < 0.05$). At HC, root:shoot ratios were similar between N treatments, and similar as well to ratios observed in HN/LC plants. Root:shoot ratios were generally greater in *B. gracilis* grown at LN, but, in contrast, no consistent treatment trends were observed in *P. smithii*. Thus, the end result of treatment combina-

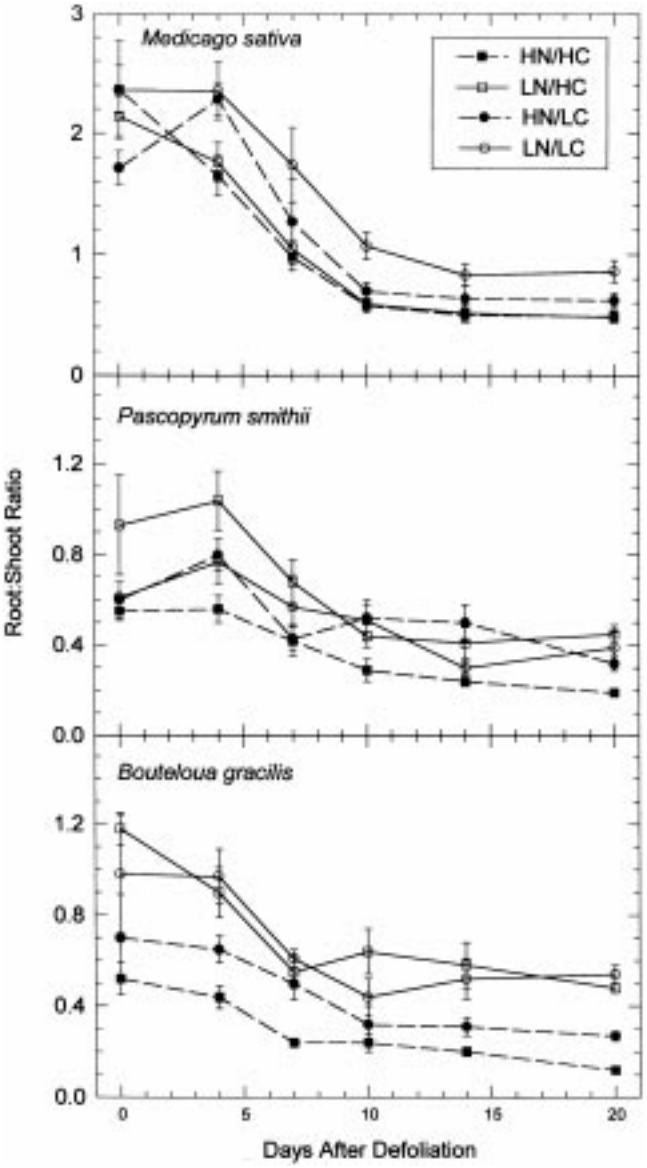

Fig. 5. Root:shoot ratio of *M. sativa*, *P. smithii*, and *B. gracilis* when grown under high/low (H/L) CO_2 and N regimes during the 20 d following defoliation. The vertical bars represent ± standard error of the mean.

tions on biomass partitioning was inconsistent, although where root:shoot ratios shifted, it was toward higher ratios under LN treatments, suggesting an increased distribution of biomass to roots in accordance with achieving a functional balance of tissue N.

The distribution of biomass between shoots and below-ground organs is homeostatic, i.e., it tends to preserve a constant relationship between the relative growth of all tissues, presumably to maintain a functional balance of resource acquisition. However, the end result of that distribution, the root:shoot ratio, is an allometric trait that varies with development and plant size, and may not provide an unambiguous measure of partitioning (Coleman et al., 1993). The analysis of treatment effects like N or CO_2 on biomass distribution and the maintenance of functional balance of plant organs are better understood in the context of the allometric growth coefficient, *k*, which "corrects" for differences associated with development and plant size (Farrar and Gunn, 1996) In *M. sativa*, the allometric growth coefficient, *k*, averaged 0.94 with no significant treatment effects (Table 2), indicating no change in partitioning due to N or CO_2 treatments. The value of *k* was close to 1, indicating similar relative growth of below- and above-ground organs at the time of measurement. So the resultant higher root:shoot ratios of LN/LC plants (Fig. 5) was apparently not due to a change in allocation strategy, but rather to retarded plant growth (Fig. 2 and 5).

Table 2. Allometric growth coefficient (*k*) values for each species, averaged across all treatment combinations for *M. sativa*, and presented only for significant treatments or treatment combinations for *P. smithii* and *B. gracilis*.

			ANOVA Results	
Species	**Treatment**	***k***	**Source**	***P* > *F***
M. sativa	all	0.94	N	ns
			CO_2	ns
			N × CO_2	ns
P. smithii	HN†	0.99b	N	0.02
	LN	0.63a		
B. gracilis	HN/HC	0.95c	N	0.002
	LN/HC	0.44a	N × CO_2	0.02
	HN/LC	0.89c		
	LN/LC	0.58b		

† Treatments are low nitrogen (LN), high nitrogen (HN), low CO_2 (LC) and high CO_2 (HC). When treatment $P \leq 0.05$, Fishers least significant difference means comparison test is shown. Means followed by different letters are statistically different at the $P \leq 0.05$ level.

In *P. smithii*, *k* averaged 0.63 for LN plants and 0.99 for HN plants, indicating 1) relative growth of roots was greater than shoots in LN plants, whereas relative growth was approximately equal between above- and below-ground organs for HN plants, and 2) LN increased biomass partitioning to belowground organs (Table 2). No significant effects of CO_2 nor interactions of CO_2 with N were detected in *P. smithii*. In *B. gracilis*, LN also significantly reduced *k*, but a N × CO_2 interaction was also observed. As a result, the coefficient *k* was greatest under HN/LC (0.89) and HN/HC (0.95), intermediate under LN/LC (0.58), and least under LN/HC (0.44) (Table 2).

Metabolites and Partitioning

Several measures of whole-plant C and N were analyzed for N and CO_2 treatment effects (Table 3). Concentrations of TNC, as well as the proportion of labile C (C_{lab}; TNC − storage carbohydrates) to N_{lab} were almost always greater in each species at HC compared with LC, suggesting that photosynthetic enhancements in all three species grown at elevated CO_2 were sufficient to elevate carbohydrate concentrations. Concentrations of whole-plant N were generally less for plants grown at HC, especially when expressed on a plant dry weight (DW) basis. The effect of CO_2 on N was sometimes less evident when expressed on a structural dry weight (SDW) basis or in terms of N_{lab}, indicating that part of the CO_2-induced drop in plant N concentration was due to carbohydrate dilution. Concentrations of N appeared

Table 3. Measures of whole-plant C and N status for 10 to 20 d after defoliation of three forage species.

Species	Treatment	TNC†	N_{lab}	Total N	Total N	N_{lab}	TNC/N	TNC/N_{lab}	C_{lab}/N_{lab}	Sucr./N_{lab}
		mg/g SDW			mg/g DW	mg/g Tot N				
M. sativa	HN/HC§	329b‡	6.8bc	26b	18b	272a	2.3b	11.7a	3.1a	1.7a
	LN/HC	429a	7.3ab	26b	17b	282a	2.8a	12.3a	2.9a	1.6a
	HN/LC	122c	7.6a	30a	25a	272a	0.8c	3.5b	1.4b	0.9b
	LN/LC	135c	6.5c	29a	24a	242b	1.0c	4.7b	1.7b	1.1b
B. gracilis	HN/HC	137a	4.6a	21a	18a	225a	1.1b	7.1b	2.6b	1.1c
	LN/HC	142a	2.1c	11c	9c	216a	2.5a	15.4a	5.4a	2.3a
	HN/LC	44b	3.9b	20a	19a	193b	0.4d	2.4c	1.5c	0.9c
	LN/LC	58b	2.2c	13b	12b	163c	0.8c	7.0b	3.2b	1.8b
P. smithii	HN/HC	86a	5.5a	21a	19ab	253a	0.7b	4.2b	2.2b	0.9c
	LN/HC	94a	3.4b	14b	13c	246a	1.3a	7.6a	4.4a	1.7a
	HN/LC	39b	3.9b	22a	20a	181b	0.3c	2.6c	2.3b	1.1bc
	LN/LC	45b	3.4b	20a	18b	179b	0.4c	3.1bc	2.8b	1.3b

† Abbreviations are total non-structural carbohydrates (TNC), labile N (N_{lab}) and C (C_{lab}) metabolites, structural dry weight (SDW), dry weight (DW), and sucrose (sucr.).
‡ When treatment $P \leq 0.05$, Fishers least significant difference means comparison test is shown. Means followed by different letters are statistically different at the $P \leq 0.05$ level.
§ Treatment designations are high N (HN), low N (LN), high CO_2 (HC) and low CO_2 (LC).

greater under LN in *M. sativa* compared with the two grass species, and the effect of N treatment on whole-plant N concentrations was more evident in the grasses. While no data were collected to indicate whether N fixation was significant enough in these *M. sativa* plants to affect their response to CO_2, it does seem likely that their N-fixing capacity, although limited in seeding-year plants, may have been an important factor in the different responses observed for the grasses and *M. sativa*.

To explore the relationship between biomass partitioning and levels of metabolites for the grass species, correlations between *k* and the C and N metabolite measures from Table 3 were investigated. No correlations were done on *M. sativa* since we could not detect a significant treatment response of *k* for this species. For *B. gracilis* alone and an analysis combining both grass species, several measures of plant N status (Total N expressed on a DW basis, Total N expressed on a SDW basis, and N_{lab} expressed on a SDW basis), and the ratio of sucrose to N_{lab} were all significantly correlated with *k* (Table 4). The signs of the correlations indicate increased structural biomass partitioning to belowground organs with either low tissue N concentrations or proportional high levels of sucrose relative to N_{lab}. In *P. smithii*, only N_{lab} on a SDW basis was significantly correlated with *k*.

In summary, no significant change in distribution strategy was detected in the N-fixer, *M. sativa*, in response to CO_2 and N treatments. Further, the magnitude and significance of changes in whole-plant N and C compounds in reaction to N and CO_2 treatments were less than observed in the two grass species. For both grass species, growth under the low N fertility regime caused significant reductions in concentrations of total N and labile N compounds, and shifted biomass partitioning in favor of below-ground organs compared with N-fertilized plants. This shift was greatest in *B. gracilis* plants grown under LN/HC, the treatment combination that resulted in the lowest whole-plant N levels in the study. These results are consistent with our hypothesis that alterations in biomass partitioning under elevated CO_2 will likely occur in the low N environment, in accordance with functional balance. We suspect the lack of a direct or interactive effect of CO_2 on partitioning in *P. smithii* was due to its low growth rate and generally higher N concentrations compared with *B. gracilis*. For CO_2 to have a significant effect on plant partitioning, growth must reach a sufficient rate in proportion to soil N supply to elicit an adaptive response. In a general sense, these results clearly indicate that any alterations in root:shoot partitioning which accompany CO_2 enrichment are contingent on other environmental factors, and are consistent with the literature which suggests increased partitioning of biomass to roots under elevated CO_2 is more likely when nutrients or water are limiting (Rogers et al., 1994, 1996). The results also underscore the inadequacy of evaluating partitioning responses soley in terms of root:shoot ratios, and the importance of considering the relative growth responses of plant organs to identify treatment-related partitioning responses.

Our motivation in evaluating several measures and proportions of labile C and N compounds was that such metabolite pools may be more closely related to parti-

Table 4. Correlations between allometric growth coefficient (*k*) and several measures of whole-plant C and N status for 10 to 20 d after defoliation of two forage species. A positive correlation indicates that partitioning to shoots increased as the particular parameter increased. A negative correlation is an indication of increased partitioning to roots.

Species	TNC‡	N_{lab}	Total N	Total N	N_{lab}	TNC/N	TNC/N_{lab}	C_{lab}/N_{lab}	Sucr./N_{lab}
	SDW basis			DW basis	Tot N basis				
B. gracilis	−0.17	0.97*	0.98*	0.99**	0.13	−0.70	−0.78	−0.87	−0.97*
P. smithii	0.17	0.94†	0.59	0.64	0.25	−0.30	−0.37	−0.74	−0.88
Grass Spp.	−0.05	0.97*	0.92†	0.95*	0.29	−0.59	−0.67	−0.81	−0.95*

*, **, † Significant at $P = 0.05$, 0.01, or 0.10, respectively.
‡ Abbreviations are total non-structural carbohydrates (TNC), labile N (N_{lab}) and C (C_{lab}) metabolites, structural dry weight (SDW), dry weight (DW), and sucrose (sucr.).

tioning responses than more traditionally and easily measured attributes like total plant tissue N concentration (Campagna and Margolis, 1989; Stulen and den Hertog, 1993). Our results did not fully support this notion. Although strong negative correlations were observed between *k* and the ratio of sucrose to N_{lab}, this was the only attribute involving carbon that was significantly related to *k*. All others were nonsignificant. Several measures of whole-plant N that were unrelated to C or C forms were significantly correlated with *k*, including the strongest correlation which was established between *k* and total-plant N expressed on a SDW basis. The lack of any treatment effects on *k* in the N-fixer, *M. sativa*, lends indirect support for the importance of N in controlling partitioning. On balance, this experiment confirms the importance of N and N metabolites in plant partitioning responses, but does not provide strong correlative evidence linking C metabolites to partitioning. This lack of evidence does not negate the importance of such C metabolite pools and their proportions in regulating plant biomass partitioning. Proof of links between metabolites measured at one or just a few points in time with growth events that occur over weeks may be difficult to accomplish. While it seems feasible that labile forms of metabolites may regulate plant partitioning responses, a very limited number of whole plant or even organ-level metabolite analyses may be insufficient to reveal more localized metabolite responses that could be involved in regulating organ relative growth responses. Indeed, there is good evidence that source and sink concentrations of sucrose are important in the regulation of plant biomass partitioning (Farrar and Gunn, 1996).

CONCLUSIONS

Our results indicate that increasing atmospheric CO_2 concentrations are likely to have important impacts on productivity and biomass partitioning in forage grasses and legumes, and that differential responses among the functional groups will undoubtably affect their performance in the field. Because this study was conducted on first-year growth of perennial forage plants in a growth chamber environment with well-watered plants, some care needs to be exercised in extrapolating these results to the field environment. But some important conclusions that pertain to the field environment can be made. The finding that regrowth and plant biomass partitioning responses to CO_2 were different among the C_3 and C_4 grasses and legume, and in accordance to our hypotheses, lends support to the usefulness of functional groupings as a strategy for understanding and summarizing plant responses to CO_2. The particular results of this study, that C_3 plants demonstrated a positive CO_2-induced regrowth response, whereas regrowth of the C_4 grass was less at elevated CO_2, and that N limitations appeared to have less effect on CO_2 responses of the legume compared with the non-N fixing grasses, indicate important generalities that could be important in the field, but need to be understood in the context of the environment. For instance, we know CO_2 enhancement can stimulate growth of C_4 grasses, especially in water-limited environments because of increased water use efficiency (e.g., Owensby et al., 1999). On the other hand, there are examples of studies in which elevated CO_2 did not elicit growth responses in second- and third-year *M. sativa* (Bunce, 1995). So the use of functional group knowledge needs to be applied carefully, because of environmental interactions with CO_2, many of which are still poorly understood. Our results also indicate that changes in plant biomass partitioning from CO_2 enrichment may be considered in terms of the balanced growth concept. Strong relationships between various plant N attributes in the grasses and biomass partitioning responses in this study indicate that whole plant measures of plant N will likely be useful for modeling and interpretive applications in situations in which plants are subjected to variable N or elevated CO_2. Finally, because of their capability to fix N, CO_2 enrichment may not be as likely to lead to increased partitioning below-ground in legumes compared with grasses, and productivity may be more responsive to elevated CO_2 compared with grasses, especially in N-limited, semiarid rangelands where the water relations benefit of elevated CO_2 is likely to be important. Field studies in which legumes can be grown in competition with other species will be needed to confirm this.

ACKNOWLEDGMENTS

We thank Barry Weaver, Dean Ackerman, Dan LeCain, Jennifer Martin, Patience Russell, and Ernie Taylor for technical assistance, and Gary Richardson for statistical advice.

REFERENCES

Amthor, J.S. 1997. Plant respiratory responses to elevated CO_2 partial pressure. p. 35–77. *In* L.H. Allen et al. (ed.) Advances in carbon dioxide effects research. ASA Spec. Publ. 61 ASA, CSSA, and SSSA, Madison, WI.

Bazzaz, F.A. 1990. The response of natural ecosystems to the rising global CO_2 levels. Annu. Rev. Ecol. Syst. 21:167–196.

Bowes, G. 1991. Growth at elevated CO_2: Photosynthetic responses mediated through rubisco. Plant Cell Environ. 14:795–806.

Bunce, J.A. 1995. Long-term growth of alfalfa and orchard grass plots at elevated carbon dioxide. J. Biogeo. 22:341–348.

Campagna, M.A., and H.A. Margolis. 1989. Influence of short-term atmospheric CO_2 enrichment on growth, allocation patterns, and biochemistry of black spruce seedlings at different stages of development. Can. J. For. Res. 19:773–782.

Campbell, B.D., and A.L. Hart. 1996. Competition between grasses and *Trifolium repens* with elevated atmospheric CO_2. p. 301–317. *In* Ch. Korner and F.A. Bazzaz (ed.) Proc. Carbon dioxide, populations and communities. Acad. Press, Inc., New York.

Coleman, J.S., K.D.M. McConhnaughay, and D.D. Ackerly. 1993. Interpreting phenotypic variation in plants. Trends Ecol. Evol. 9:187–191.

Davidson, R.L. 1969. Effect of root/leaf temperature differentials on root/shoot ratios in some pasture grasses and clover. Ann. Bot. (London) 33:561–569.

Farrar, J.F., and S. Gunn. 1996. Effects of temperature and atmospheric carbon dioxide on source-sink relations in the context of climate change. p. 389–406. *In* E. Zamski and A.A. Schaffer (ed.) Photoassimilate distribution in plants and crops. Marcel Decker Pub., New York.

Farrar, J.F., and M.L. Williams. 1991. The effects of increased atmospheric carbon dioxide and temperature on carbon partitioning, source-sink relations and respiration. Plant Cell Environ. 14: 819–830.

Gonzàlez-Meler, M.A., M. Ribas-Carbó, J.N. Siedow, and B.G. Drake. 1996. Direct inhibition of plant mitochondrial respiration by elevated CO_2. Plant Physiol. 112:1349–1355.

Harris, W. 1978. Defoliation as a determinant of the growth, persistence and composition of pasture. p. 67–85. *In* J.R. Wilson (ed) Plant relations in pastures. CSIRO, East Melbourne, Australia.

Hebeisen, T., A. Lüschler, S. Zanetti, B.U. Fischer, U.A. Hartwig, M. Frehner, G.R. Hendrey, H. Blum, and J. Nösberger. 1997. Growth response of *Trifolium repens* L. and *Lolium perenne* L. as monocultures and bi-species mixture to free air CO_2 enrichment and management. Global Change Biol. 3:149–160.

Hunt, H.W., E.T. Elliott, J.K. Detling, J.A. Morgan, and D.-X. Chen. 1996. Responses of a C_3 and a C_4 perennial grass to elevated CO_2 and temperature under different water regimes. Global Change Biol. 2:35–47.

Hunt, H.W., J.A. Morgan, and J.J. Read. 1998. Simulating growth and root-shoot partitioning in prairie grasses under elevated atmospheric CO_2 and water stress. Ann. Bot. (London) 81:489–501.

Hunt, R., D.W. Hand, M.A. Hannah, and A.M. Neal. 1995. Temporal and nutritional influences on the response to elevated CO_2 in selected British grasses. Ann. Bot. (London) 75:207–216.

Lauenroth, W.K., and D.G. Milchunas. 1991. Short-grass steppe. p. 183–226. *In* R.T. Coupland (ed.) Ecosystems of the world 8A: Natural grasslands, introduction and western hemisphere. Elsevier, New York.

LeCain D.R., and J.A. Morgan. 1998. Growth, gas exchange, leaf nitrogen and carbohydrate concentrations in NAD-ME and NADP-ME C_4 grasses in elevated CO_2. Phys. Planta. 102:297–306.

Lüscher, A., T. Hebeisen, S. Zanetti, U.A. Hartwig, H. Blum, G.R. Hendrey, and J. Nösberger. 1996. Differences between legumes and nonlegumes of permanent grassland in their responses to free-air carbon dioxide enrichment: Its effect on competition in a multispecies mixture. p. 287–300. *In* Ch. Korner and F.A. Bazzaz (ed.) Carbon dioxide, populations and communities. Acad. Press, Inc., New York.

Milchunas, D.G., and W.K. Lauenroth. 1993. Quantitative effects of grazing on vegetation and soils over a global range of environments. Ecol. Monog. 63:327–366.

Morgan, J.A., H.W. Hunt, C.A. Monz, and D.R. LeCain. 1994. Consequences of growth at two carbon dioxide concentrations and temperatures for leaf gas exchange of *Pascopyrum smithii* (C_3) and *Bouteloua gracilis* (C_4). Plant Cell Environ. 17:1023–1033.

Owensby, C.E., P.I. Coyne, J.M. Ham, L.M. Auen, and A.K. Knapp. 1993. Biomass production in a tallgrass prairie ecosystem exposed to ambient and elevated CO_2. Ecol. Applic. 3:644–653.

Owensby, C.E., J.M. Ham, A.K. Knapp, and L.M. Auen. 1999. Biomass production and species composition change in a tallgrass prairie ecosystem after long-term exposure to elevated atmospheric CO_2. Global Change Biol. 5:497–506.

Poorter, H. 1993. Interspecific variation in the growth response of plants to an elevated ambient CO_2 concentration. Vegetatio 104/105:77–97.

Richards, J.H., and M.M. Caldwell. 1985. Soluble carbohydrates, concurrent photosynthesis and efficiency in regrowth following defoliation: A field study with *Agropyron* species. J. Appl. Ecol. 22: 907–920.

Rogers, H.H., S.A. Prior, G.B. Runion, and R.J. Mitchell. 1996. Root to shoot ratio of crops as influenced by CO_2. Plant Soil 187:229–248.

Rogers, H.H., G.B. Runion, and S.V. Krupa. 1994. Plant responses to atmospheric CO_2 enrichment with emphasis on roots and the rhizosphere. Environ. Pollut. 83:155–189.

Ryle, G.J.A., and C.E. Powell. 1992. The influence of elevated CO_2 and temperature on biomass production of continuously defoliated white clover. Plant Cell Environ. 15:593–599.

Sæbø, A., and L.M. Mortensen. 1995. Growth and regrowth of *Phleum pratense*, *Lolium perenne*, *Trifolium repens* and *Trifolium pratense* at normal and elevated CO_2 concentration. Agric. Ecosys. Environ. 55:29–35.

Schapendonk, A.H.C.M., P. Dijkstra, J. Groenwold, C.S. Pot, and S.C. Van De Geijn. 1997. Carbon balance and water use efficiency of frequently cut *Lolium perenne* L. swards at elevated carbon dioxide. Global Change Biol. 3:207–216.

Schenk, U., H.-J. Jäger, and H.-J. Weigel. 1997. The response of perennial ryegrass/white clover swards to elevated atmospheric CO_2 concentrations. 1. Effects on competition and species composition and interaction with N supply. New Phytol. 135:67–79.

Skinner, R.H., J.A. Morgan, and J.D. Hanson. 1999. Carbon and nitrogen reserve remobilization following defoliation: Nitrogen and elevated CO_2 effects. Crop Sci. 39:1749–1756.

Stulen, I., and J. den Hertog. 1993. Root growth and functioning under atmospheric CO_2 enrichment. Vegetatio 104/105:99–115.

Troughton, A. 1956. Studies on the growth of young grass plants with special reference to the relationship between the shoot and root systems. J. Brit. Grassl. Soc. 11:56–65.

Wand, J.E., G.F. Midgley, M.H. Jones, and P.S. Curtis. 1999. Responses of wild C_4 and C_3 grass (Poaceae) species to elevated atmospheric CO_2 concentration: a meta-analytic test of current theories and perceptions. Global Change Biol. 5:723–741.

Wilsey, B.J. 1996. Urea additions and defoliation affect plant responses to elevated CO_2 in a C_3 grass from Yellowstone National Park. Oecologia 108:321–327.

Wilsey, B.J., J.S. Coleman, and S.J. McNaughton. 1997. Effects of elevated CO_2 and defoliation on grasses: A comparative ecosystem approach. Ecol. Applic. 7:844–853.

Wullschleger, S.D., L.H. Ziska, and J.A. Bunce. 1994. Respiratory responses of higher plants to atmospheric CO2 enrichment. Physiol. Plant. 90:221–229.

Zanetti, S., U.A. Hartwig, A. Lüscher, T. Hebeisen, M. Frehner, B.U. Fischer, G.R. Hendrey, H. Blum, and J. Nösberger. 1996. Stimulation of symbiotic N_2 fixation in *Trifolium repens* L. under elevated atmospheric pCO_2 in a grassland ecosystem. Plant Physiol. 112:575–583.